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Coral Endolithic Algae: Life in a Protected Environment[1]

N. SHASHAR,[2] A. T. BANASZAK,[3] M. P. LESSER,[4] AND D. AMRAMI[5]

ABSTRACT: Endolithic algae inhabiting skeletons of living corals appear to be adapted to an extreme environment created by the coral. However, measurements on three coral species from the genus *Porites* revealed that these corals provide several modes of protection to the algae as well. High concentrations of ultraviolet (UV)-absorbing compounds, mycosporine-like amino acids (MAAs), were found in the tissues of all corals examined, but they were not detected in extracts of the endolithic algae. Coral tissues and skeleton filter 93.98–99.5% of the ambient UV radiation and thus shade the endolithic algae from this potentially damaging radiation. In addition endolithic algae are largely relieved from grazing pressure by herbivorous fish, because only 4% of fish bites on *Porites* corals resulted in exposed endolithic algae. Thus, the coral skeleton provides a refuge to the endolithic algae from some of the environmental pressures normally experienced by free-living algae on the reef.

THE CALCIUM CARBONATE skeleton of living corals provides a unique habitat for both algae and bacteria (Odum and Odum 1955). These "endolithic" organisms have been described from numerous scleractinian corals (Shashar and Stambler 1992), as well as the hydrozoan *Millepora tenella* Ortmann (Bellamy and Risk 1982). In most cases, these endolithic organisms include filamentous algae, usually siphonaceous chlorophytes of the genus *Ostreobium* (Duerden 1902, Jeffrey 1968, Lukas 1974), which easily can be seen as a green band or zone when the coral is broken. According to Campion-Alsumard et al. (1995), *Ostreobium quekettii* Bornet & Flahault is the only chlorophyte species known to survive in the unique environment created by a living coral. Among the corals hosting endolithic algae are several species from the genus *Porites: P. compressa* Dana (Shashar and Stambler 1992), *P. evermanni* Vaughan (N.S., pers. obs.), *P. lobata* Dana (MacIntyre and Town 1975, Patzol 1988), and *P. lutea* Edwards & Haime (Highsmith 1979, 1981). In the genus *Porites,* these algae may appear as dense green bands underneath the coral tissue, as in *P. lobata* and *P. evermanni,* or can be found throughout the coral skeleton, as in the branching *P. compressa.*

Odum and Odum (1955) hypothesized a major contribution by the endolithic algae to the primary productivity of the reef. This suggestion was later challenged by Kanwisher and Wainwright (1967) and by Shashar and Stambler (1992), who reported a photosynthetic rate of 0.01 mg $O_2 \cdot min^{-1} \cdot$ ml coral skeleton^{-1} for *P. compressa.* This low rate of photosynthesis can be attributed to strong attenuation of solar radiation by the coral tissue, composed of cnidarian host and algal symbionts (Halldal 1968, Shibata and Haxo 1969), and by the inorganic coral skeleton (Kanwisher and Wainwright 1967).

Coral reefs present various types of environmental pressures for algae growing on them. These include competition for substrate with other sessile species, grazing by numerous herbivorous fish, photoinhibitory and other damaging effects of solar radiation, both photosynthetically active radiation (PAR) and ultravi-

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[2] Marine Biology Laboratory, Woods Hole, Massachusetts 02543.

[3] Department of Biological Sciences, University of California, Santa Barbara, California 93106. Current address: Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, Maryland 21037.

[4] Department of Zoology, University of New Hampshire, Durham, New Hampshire 03824.

[5] Department of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel.

olet (UV) radiation. Living within coral skeletons relieves some of the intraspecific competition for space and possibly provides refuge from grazing and potentially damaging effects of solar radiation.

Surviving within the skeleton of living corals requires specific adaptations by the endolithic algae (Shashar and Stambler 1992). These include adaptation to low PAR, to diurnal fluctuations in pH and oxygen concentrations, and to limited exchange of both solutes and particulate matter with the water column. On the other hand, regeneration of nutrients (Risk and Muller 1983, Ferrer and Szmant 1988) along with nitrogen fixation (Shashar et al. 1994) by other members of the endolithic community may provide a nutrient source to the algae.

Corals are known to possess several compounds that absorb solar radiation at various wavelengths. These include not only the pigments involved in the photosynthetic process but also compounds that are believed to provide protection from damage by UV radiation. These compounds, called mycosporine-like amino acids (MAAs) (formerly known as "S-320" [Shibata 1969, Dunlap et al. 1986]), are water-soluble, nitrogenous substances, which maximally absorb light in the range of 310–360 nm. By absorbing across the UV-A and UV-B spectrum, these compounds have been hypothesized to protect UV-sensitive cellular compounds from the damaging effects of UV radiation (Shibata 1969). However, the exact absorbance spectra of the MAAs within the tissues of living organisms are yet unknown. MAAs have been found in diverse species of marine organisms ranging from cyanobacteria (Shibata 1969) to teleosts (Dunlap et al. 1989). All coral species studied to date contain MAAs (M. Ondrusek, pers. comm.) including mycosporine-glycine (λ max 310), palythine (λ max 320), and palythinol (λ max 332) (Dunlap et al. 1986).

In a previous study (Shashar and Stambler 1992) the life history of the endolithic algae was described as one of survival in an extreme environment. In the study reported here we examined the potential advantages provided by the coral to endolithic algae residing within its skeleton.

MATERIALS AND METHODS

Colonies of the massive corals *Porites lobata* (purple morph), *P. evermanni* (yellow morph), and of the branching coral *P. compressa* (Maragos 1977) were collected from a reef flat, 2 m deep, in Kāne'ohe Bay, O'ahu, Hawai'i (21° 26′ N, 157° 47′ W), and transported to the laboratory in seawater.

Field Observations

Fish bite marks on *Porites* coral colonies (all of which contained endolithic algae) were observed while diving. Only fresh bite marks were recorded, and for each of them we recorded whether the bite mark reached below the coral tissue into the skeleton, reached below the coral tissue down into the endolithic algal zone, or whether it was restricted to the coral tissue.

Light Penetration Measurements

Live coral colonies were sliced into thin layers containing only, yet all, the coral tissue layer. Tissue depth and hence slice width were 2.98 $\pm$ 0.42 mm in *P. compressa,* 2.88 $\pm$ 0.35 mm in *P. evermanni,* and 3.68 $\pm$ 0.32 mm in *P. lobata* (mean $\pm$ SD). Colonies were handled carefully to minimize stress to the coral polyps. Slices were scanned for transmittance of PAR and UV in a UV-Vis scanning spectrophotometer (Shimadzu UV-2101 PC) with an integrating sphere attachment (LISR-2100 [UV-Vis]) over a range of 300–700 nm, in 2-nm intervals, with a slit width of 5 nm, scanning a surface area of the coral colonies of 10.4 mm^{-2} in *P. compressa,* 13.6 mm^{-2} in *P. evermanni,* and 12.5 mm^{-2} in *P. lobata.* This system enables measurements of living specimens held within seawater. Using an integrating sphere we could measure all light transmitted through the sample, even when it was diffracted or scattered from its original path. Baseline measurements were performed using the same setup as measurements (including seawater, samples holder, appropriate scanned area, etc.) but without the corals. For further description of the system see Beach et al. (1995, in press). Coral colonies were positioned, in seawater, perpendicular to the measuring beam. Coral

polyps, which were often extended before and after measurements, were gently touched so that they would be retracted, providing a comparable surface structure between species that resembles the natural state during daytime. For each of the three coral species, layers were taken from three different coral colonies, and each layer was scanned four times, to provide an average transmittance factor based on 12 scans.

Down-welling irradiance was measured on a clear day, with calm sea, at noon, at 2-m depth with a spectroradiometer (LiCor LI1800UW) equipped with a 2π, cosine-corrected sensor. The spectroradiometer was placed in a portion of the reef dominated by *P. compressa* at the depth of collection. For further descriptions and accuracy limitations of the spectroradiometer see Kirk et al. (1994). The spectroradiometer was calibrated for wavelength and irradiance ($W \cdot m^{-2} \cdot nm^{-1}$) accuracy within 3 months of our study by LiCor Inc. and checked before field measurements against the mercury lines from fluorescent lights. Measurements were taken at 2-nm intervals at a range of 300–850 nm. Readings of three scans at each wavelength were averaged to minimize flicker and wave effects.

By multiplying the transmittance factor of the coral slices by the down-welling irradiance spectra, we were able to calculate the solar spectra reaching the endolithic algae.

Laboratory Assays

Samples for laboratory assays were obtained from freshly collected corals. Tissue material was collected by cutting out thin layers containing tissue only and cutting circular samples, 11 mm in diameter (surface area of 0.95 cm^2), out of these layers. Endolithic algal material was obtained by removing coral tissue, using the water pik technique (Johannes and Weibe 1970), and then cutting the skeleton, well below the original coral tissue, into thin slices. From these slices, cores 11 mm in diameter (surface area of 0.95 cm^2), were obtained. Separate cores, with equal surface area, were used for extraction of MAAs.

MAAs were extracted in 100% high-performance liquid chromatography (HPLC)–grade methanol overnight at 4°C and quantified using reverse-phase HPLC. MAAs were separated using a Brownlee RP-8 column (Spheri-5, 4.6 mm i.d. by 25 cm) protected with an RP-8 guard column (Spheri-5, 4.6 mm i.d. by 5 cm) and an aqueous mobile phase with 40% methanol and 0.1% acetic acid (vol.:vol.). Peak detection was by UV absorption at 313 and 340 nm, calibrated against known standards, and quantification of MAAs was determined using peak area integrations at 313 nm.

RESULTS AND DISCUSSION

Living corals create a "challenging environment" to algae boring into their skeletons (Campion-Alsumard et al. 1995). Indeed only a single species of alga, *O. quekettii,* is known to meet this challenge (Campion-Alsumard et al. 1995). Incoming solar radiation is strongly attenuated by coral tissue (Halldal 1968, Shibata and Haxo 1969) (Figure 1). One should note the low transmittance in the 650- to 680-nm range caused by absorption by chlorophyll *a,* whereas combinations of peridinin, carotenoides, and chlorophylls *a* and *c* have most likely caused the broad absorption at the 400- to 500-nm range (Kuhl et al. 1995). By multiplying the down-welling irradiance by the transmittance spectrum through the coral tissue we calculated the solar radiation spectra to which the endolithic algae are exposed. In the PAR range (400–700 nm) the integrated energy reaching the endolithic algae was 2.87 $W \cdot m^{-2}$ (1.16% of ambient) in *P. compressa,* 10.86 $W \cdot m^{-2}$ (4.41% of ambient) in *P. evermanni,* and 5.42 $W \cdot m^{-2}$ (2.2% of ambient) in *P. lobata.* The tissues of the *Porites* corals transmit more PAR than the 0.1–0.6% reported by Halldal (1968) and by Shibata and Haxo (1969) for *Favia* corals. This variation is possibly due to differences in the tissue thickness between the two corals and to high pigment concentration in the "dark chocolate brown" *Favia* colonies. These low PAR intensities limit the photosynthetic rate of the endolithic algae (Kanwisher and Wainwright 1967). Shashar and Stambler (1992) reported respiration and photosynthesis rates from endolithic algae in *P. compressa* that are 1.4% of those of the coral's zooxanthellae, corresponding with the fraction of PAR reaching the endolithic algae. Halldal (1968) found that the algae cope with the strong

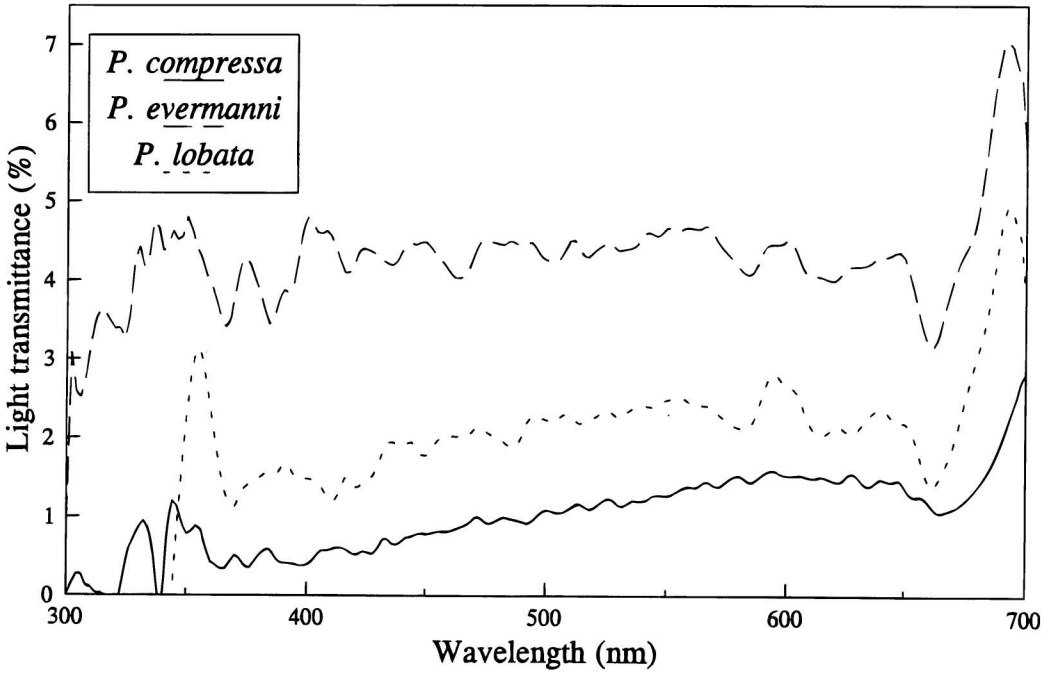

FIGURE 1. Percentage transmittance of UV and PAR through coral tissue of several *Porites* corals. Averages of 12 scans per species (one slice from each of three different colonies was scanned four times) are presented. SD were 0.02–3.7% for *P. compressa,* 0.8–3.94% for *P. lobata,* and 0.3–5.56% for *P. evermanni.*

attenuation of light by chlorophyll *a* by utilizing light in the 700- to 750-nm portion of the spectrum, which is less absorbed by the coral algal symbionts (zooxanthellae).

Coral tissues contain compounds, such as MAAs, that absorb UV radiation. The penetration of ambient UV radiation through the tissue of each coral species was calculated to be lower than PAR (Figure 2) and was 0.14 W · m^{-2} (0.5% of ambient) in *P. compressa,* 1.12 W · m^{-2} (4.02% of ambient) in *P. evermanni,* and 0.35 W · m^{-2} (1.27% of ambient) in *P. lobata* when integrated throughout the 300- to 400-nm range.

All three coral species contained high concentrations of MAAs (Table 1) at rates comparable to those of free-living algae from shallow waters (Banaszak et al. 1996). These UV-absorbing compounds may help to protect the coral from damage by the UV portion of the solar spectrum. The types and concentrations of MAAs varied between the different coral species. However, in all cases we were not able to detect any MAAs in the endolithic algae layer, even when extracted into low volumes of methanol and examined without dilution. The strong attenuation by the coral tissue limits the amount of UV radiation reaching the endolithic algae. Indeed, unlike numerous other free-living algae on the reef (Banaszak et al. 1996), the endolithic algae do not contain any of these UV-absorbing compounds. As of yet, it is not clear whether the endolithic algae can produce MAAs but have not been induced to synthesize them because of the low doses of UV radiation, or whether they lack the ability to produce MAAs and are limited to protected niches such as coral skeletons.

Grazing is yet another factor affecting algae in the reef. However, endolithic algae are largely relieved of this pressure. Fish feeding on coral tissue by biting into the tissue and/or skeleton usually do not reach the endolithic algae region under the tissue. Field observations revealed that in most cases (96 out of 100 observations) fish bites do not penetrate through the coral tissue and therefore do not reach the endolithic algae.

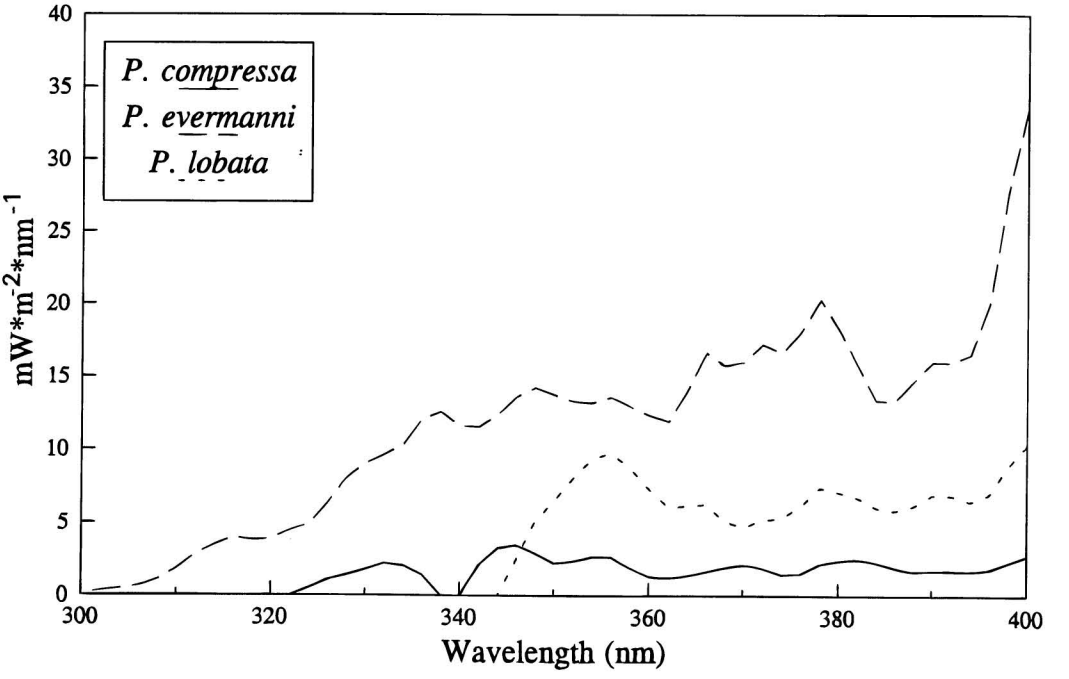

FIGURE 2. Amount of solar UV radiation reaching the endolithic algae. Using downwelling irradiance measurements and the percentage transmittance through the coral, we calculated the radiation intensity reaching the endolithic algae.

Only in four cases were the endolithic algae exposed, and only in two of these were the endolithic algae layers penetrated and the inner coral skeletons exposed. Therefore, the endolithic algae are protected from grazing by the coral skeleton, and even when the coral tissue is eaten they remain protected from UV radiation damage.

Algae living within the skeleton of a living coral exist in a unique and challenging environment. However, the coral provides them with several categories of protection that allow the endolithic algae to exploit this unique habitat successfully.

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TABLE 1

MAAs CONCENTRATIONS IN THREE SPECIES OF *Porites* CORALS

MAA NAME	λ max (nm)	CORAL SPECIES		
		P. lobata	*P. evermanni*	*P. compressa*
Mycosporine-glycine	310	7.22 ± 2.59	48.50 ± 8.14	4.96 ± 0.15
Palythine	320	1.16 ± 0.40	0.054 ± 0.079	0.0
Palythinol	332	1.75 ± 0.57	0.0	9.25 ± 0.41
Shinorine	334	6.51 ± 1.67	7.63 ± 0.46	1.87 ± 0.43

NOTE: nmol MAA · cm^{-2} surface area, mean ± SD, $n = 3$ for each species.

UV-absorbing compounds. MAAs standards were kindly provided by Walter Dunlap.

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